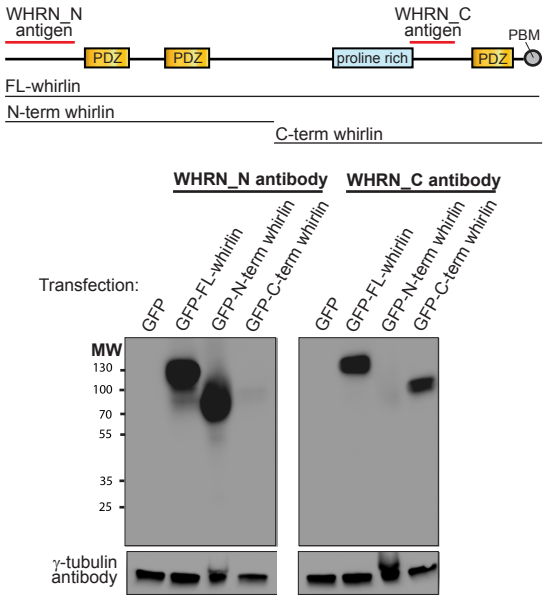
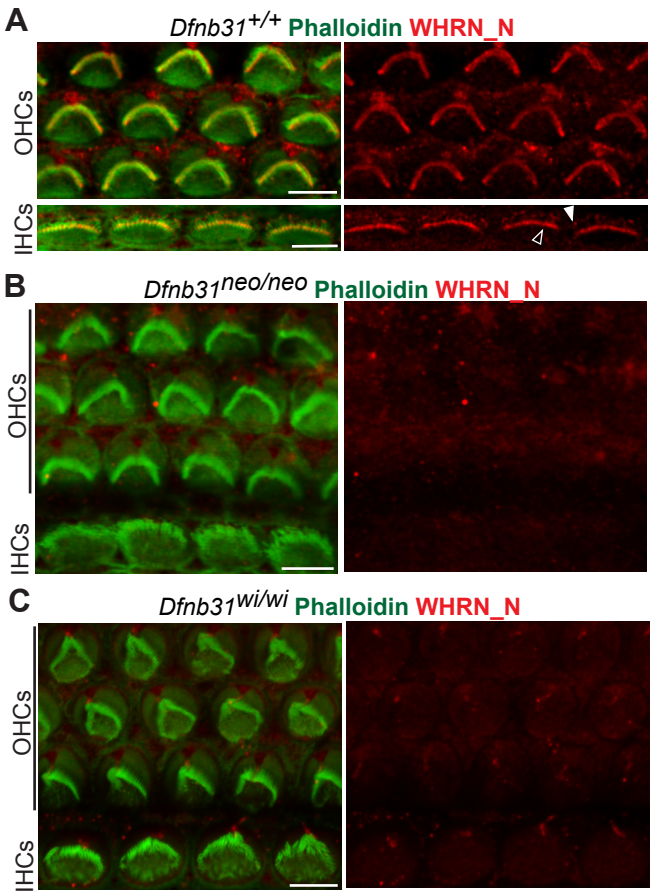
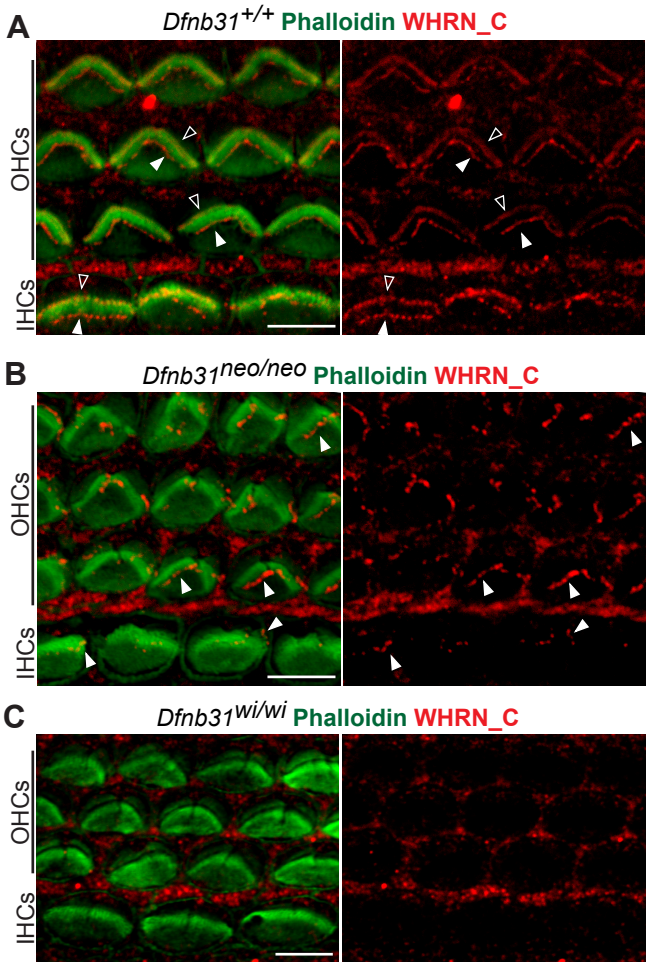

WHRN_N antigen
WHRN_C antigen
PBM
PDZ
PDZ
proline rich
PDZ
FL-whirlin
N-term whirlin
C-term whirlin
WHRN_N antibody
WHRN_C antibody
Transfection:
GFP
GFP-FL-whirlin
GFP-N-term whirlin
GFP-C-term whirlin
GFP
GFP-FL-whirlin
GFP-N-term whirlin
GFP-C-term whirlin
MW
130
100
70
55
35
25
γ-tubulin antibody


A
Dfnb31+/+ Phalloidin WHRN_N
OHCs
IHCs
B
Dfnb31neo/neo Phalloidin WHRN_N
OHCs
IHCs
C
Dfnb31wi/wi Phalloidin WHRN_N
OHCs
IHCs


A
Dfnb31+/+ Phalloidin WHRN_C
OHCs
IHCs
B
Dfnb31neo/neo Phalloidin WHRN_C
OHCs
IHCs
C
Dfnb31wi/wi Phalloidin WHRN_C
OHCs
IHCs

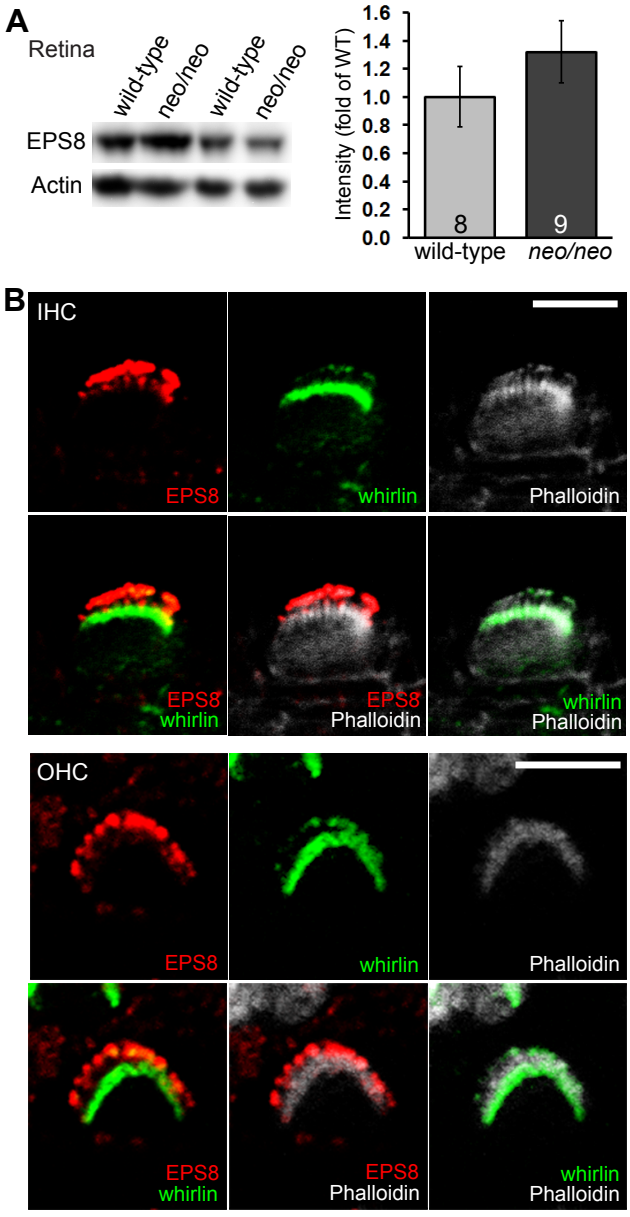

A
Retina
wild-type
neo/neo
wild-type
neo/neo
EPS8
Actin
Intensity (fold of WT)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
8
9
wild-type
neo/neo
B
IHC
EPS8
whirlin
Phalloidin
EPS8
whirlin
EPS8
Phalloidin
whirlin
Phalloidin
OHC
EPS8
whirlin
Phalloidin
EPS8
whirlin
EPS8
Phalloidin
whirlin
Phalloidin

Figure S1. Confirmation of the whirlin antibodies' specificity. Rabbit WHRN_N antibody was able to detect GFP-fused whirlin full-length (FL-whirlin) and N-terminal (N-term whirlin) fragments, but not GFP or GFP-fused whirlin C-terminal (C-term whirlin) fragments. On the other hand, rabbit WHRN_C antibody was able to detect GFP-fused whirlin full-length and C-terminal fragments, but not GFP or GFP-fused whirlin N-terminal fragments. GFP and GFP-fused whirlin fragments were expressed in transfected HEK293 cells. Anti-γ tubulin blots were shown as a control for sample loading and integrity.

Figure S2. Low-magnification views of wild-type, *Dfnb31*$^{neo/neo}$ and *Dfnb31*$^{wi/wi}$ cochleas stained by rabbit WHRN_N antibody. Empty arrow, stereociliary base; white arrow, stereociliary tip; red signals outside stereociliary bundles, non-specific; scale bars, 5 μm.

Figure S3. Low-magnification views of wild-type, *Dfnb31*$^{neo/neo}$ and *Dfnb31*$^{wi/wi}$ cochleas stained by rabbit WHRN_C antibody. Empty arrows, stereociliary base; white arrows, stereociliary tip; red signals outside stereociliary bundles, non-specific; scale bars, 5 μm.

Figure S4. EPS8 expression in the retina and colocalization of EPS8 and whirlin in cochlear hair cells. (**A**) Immunoblotting analysis showed that EPS8 protein expression did not change in *Dfnb31*$^{neo/neo}$ retinas, compared with wild-type retinas. Left, representative immunoblots. The blot for actin signals was loading control. Right, quantification of EPS8 signal intensities on immunoblots. Numbers at the bottom of bars are numbers of mice examined in groups. Error bars, standard error of the mean. Student's *t* tests (two-tail) were performed. (**B**) Double

immunostaining showed that EPS8 (red) and whirlin (green) colocalized at the tip of stereocilia (phalloidin, white) in both P4 wild-type IHCs (upper two rows) and OHCs (lower two rows).

Table S1. PCR primers used in studies on *Dfnb31* mRNA variant expressions.

Primer Name	Primer Sequence
a	CCAGCGCTCTAGCTCACCTGC
b	GCTCTAGCTCACCTGCACCT
c	GGAGGTGCGACTCGTGAGCC
d	GTTAACCAGGGCCAGGTGGGTGTC
e	GGCCCCACCCCTAGAGCATC
f	GTAGGAGATGCGAGCACTTTGTACGC
g	TATCCCCAGACTGAGAGCCCCAGAA
h	TATAAGGTGGTGGCCAGGAGAC
i	TCCACAGACAGGATGATGGC